

DIETARY IMMUNOASSAY OF *ILYANASSA OBSOLETA*, THE EASTERN MUD SNAIL*

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ABSTRACT

The gut contents of mud snails from a high intertidal saltmarsh in South Carolina were examined both visually and immunologically from eight sample dates. Antisera to a variety of potential prey types were used in double-immunodiffusion tests of the amorphous gut material. Near absence of meiofaunal prey and presence of scant animal remains confirms the facultative carnivorous feeding mode for this species, while presence of frustules, sediments, and detritus indicated the dominance of herbivory and detritivory. *Ilyanassa obsoleta* is probably relatively unimportant as a predator on living benthic invertebrates, but it may be very important in detrital remineralization and physical breakdown processes.

INTRODUCTION

Feeding studies of the eastern mud snail, *Ilyanassa obsoleta* (Say), have provided nearly as many characterizations of its feeding mode as the number of studies conducted. It has been called primarily a deposit feeder (Pace *et al.*, 1979), an omnivorous deposit feeder (Nichols and Robertson, 1979), a facultative carrion-feeder (Gurin and Carr, 1971), a non-selective biological 'vacuum cleaner' (Curtis and Hurd, 1981), or a facultative herbivore/carnivore rather than an omnivore (Brown, 1969). Sheltema (1964), however, regarded the snail as principally herbivorous. Conner and Edgar (1982) found that living diatoms comprised a major portion of the snails' diet and surmised that dead foods were unimportant. It is remarkable that visual analysis of this common mollusc's gut contents could generate so many descriptions of its trophic mode. Much of the variability may stem from habitat heterogeneity or time of collection (see Robertson, 1979), but more likely than not, much of the classification difficulty derives from our inability to identify gut contents with traditional visual techniques.

Studies such as the above are typical for the gut contents analysis of marine benthic deposit feeders in that even qualitative estimates of the relative importance of different foods are made with little assurance of their accuracy. Direct observations of ingestion are nearly impossible with deposit-feeders, so we have had to rely on visual analysis of gut contents for dietary information. Despite the drawbacks of microscopical identifications, most of our knowledge of trophic interactions is derived from such studies. As part of a larger study of trophic connections among salt marsh benthic invertebrates, the stomach contents of *I. obsoleta* were examined using the immunological methods of Feller *et al.* (1979) which were designed to identify the soluble proteins in gut contents that could not be identified using traditional visual methods. Although subject to many of the same biases as visual analysis (*e.g.*, dif-

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ferential rates of digestion for different prey types, ingestion not necessarily implying assimilation, etc.), the immunological technique allows positive identification of prey when visual techniques do not. Furthermore, negative results with the immunological method are also informative in that they allow one to state that various prey types were not present in a particular gut sample. With visual analysis alone, one cannot state explicitly what was absent in the sample. Absence in this sense is determined by the lower limit of sensitivity of the immunological method.

Ilyanassa obsoleta were sampled periodically during the year to provide gut material on which parallel visual and immunological assays could be made. It was hoped that the immunoassays would provide data to test the hypotheses that mud snails eat meiofauna (Pace *et al.*, 1979), that *Spartina* detritus is not utilized directly (Wetzel, 1977), and that carnivory is facultative rather than obligatory for this species.

MATERIALS AND METHODS

Mud snails were collected at random during low tide from the high intertidal saltmarsh near Oyster Landing at North Inlet, South Carolina, (33°20'N, 70°10'W) on eight occasions from April, 1980, to June, 1981. The marsh is a typical southeastern *Spartina alterniflora* Loisel habitat which is covered to a depth of approximately 0.3 m twice per tidal day. On each sampling occasion, approximately 30 snails were sampled and frozen in the field on dry ice. Lots of five snails in the size range 15–19 mm total shell length were pooled for analysis. Within each five-snail lot, individuals were dissected and their entire digestive tract removed with forceps. Each gut was examined as a smear on a glass microscope slide with the aid of a dissecting microscope at 500× magnification. The guts of these five snails were then pooled and solubilized in 0.1 ml TES-saline [5 mM N-tris (hydroxymethyl) methyl-2-aminoethane sulfonic acid, 30 mM NaOH, and 150 mM NaCl] at pH 7.3. Thus the same material was examined both visually and immunologically.

For immunoassay, 20 μ l of solubilized gut material was placed centrally in a plastic template on agarose surrounded by small wells containing antisera to potential prey utilizing the micro-Ouchterlony technique (Ouchterlony, 1968). Precipitin lines which formed between the wells by diffusion of solubilized gut material and peripheral antisera were counted. Each gut preparation was assayed in duplicate with antisera to 19 different potential prey present in the study area. Cross-reactions and homologous self-reactions between solubilized whole-organism extracts of each potential prey item and the entire antibody array are shown in Table I. Antiserum preparation followed Feller *et al.* (1979). No attempt was made to assay the snail guts for the presence of large, rare organisms that occur in the high marsh because either no antiserum was available at the time or it was deemed too unlikely that mud snails preyed on them alive. These included blue crabs, (*Callinectes sapidus*), burrowing ghost shrimp of the genus *Callianassa*, wharf crabs (*Sesarma* spp.), and various fishes. The small volumes of gut material present in the snails also precluded any attempts to standardize the total soluble protein content of each batch prior to immunoassay.

The algorithm described by Feller *et al.* (1979) was used to confirm the presence/absence of specific prey. An example of how this algorithm operates is shown in Table II using a hypothetical community of just six species. Since antiserum to a given prey organism may also produce precipitin lines with proteins from the predator's gut lining and/or different prey in the gut, the algorithm was designed to mathematically eliminate all precipitin line formations which could have resulted from these cross-reactions. The conservative nature of this algorithm thus provides a minimum estimate of the true number of different prey taxa consumed by a predator. Additional details of the algorithm have been discussed by Feller *et al.* (1979 [p. 67]).

TABLE I
Maximum number of precipitin lines observed in extract-antiserum immunodiffusion cross-reaction tests

Antisera to:	Whole-Organism extracts																			
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
A	15	1	6	10		8	7	1	1	1	2	2	2	2	2	3			1	1
B	7	1	2																	
C	3	2	11	10		4	4						2	1	1					
D	9	1	8	14		4	6		2	5	2	3			3	2		1	2	2
E	5	1	2	5	7	4	3	1	2	2	1	2	3	1	1			1	2	
F	4	1	2	5		13	5		1	2	1	1				2				
G	5	5	2	2		8	8	1		1		1								1
H	1	2	2	1		1	1	11	7	2	4	4	4	2	2	2	2	2	2	1
I	1	1		2	1	2	2	5	12	3	4	7	3	4	3	2	2	2	5	
J	2		1	1		2	3	3	9	6	3	4	6	6	3	3	2	2	3	1
K	1	2		3	1	2	2	6	4	8	13	8	7	9	5	6	3	3	8	
L	2	2	2	3		3	3	4	7	5	8	15	4	6	5	2	2	4	4	
M	2	4		4		2	5	3	5	3	6	7	12	5	6	5	2	3	3	2
N	1	1	2	3		2	3	3	2	4	5	7	7	12	6	3	1	1	6	
O	1	1	2	2		2	2	4	4	3	3	7	9	5	14	1	1	4	4	
P	1	1	2	1		2	2	2	2	2	2	2	2	2	3	12	2	2	3	1
Q				1			3	1		1				1	1	1	6		1	
R				1					1	1								6		
S				1	1	4	3	2	3	4	2	5	4	4	3	4	2	2	11	
T				1																8

A = *Penaeus setiferus* (white shrimp)
 B = Harpacticoid copepods
 C = *Uca pugnax* (fiddler crab)
 D = *Uca pugilator* (fiddler crab)
 E = Ostracods
 F = *Palaemonetes pugio* adults (grass shrimp)
 G = *Palaemonetes pugio* juveniles (grass shrimp)
 H = *Crassostrea virginica* spat (oyster)
 I = *Crassostrea virginica* adult (oyster)
 J = *Mercenaria mercenaria* spat (hard clam)
 K = *Mercenaria mercenaria* adult (hard clam)
 L = *Geukensia demissa* (mussel)
 M = *Littorina irrorata* (periwinkle)
 N = *Tagelus plebeius* (razor clam)
 O = *Ilyanassa obsoleta* (mud snail)
 P = Oligochaetes
 Q = Nematodes
 R = Turbellaria
 S = *Diopatra cuprea* (polychaete)
 T = *Spartina alterniflora* (marsh cordgrass)

Italicized numbers on diagonal refer to self-reactions. Blanks denote no cross-reaction. These data are equivalent to Table II, part A.

TABLE II

Hypothetical example using the immunoassay algorithm

		Whole-organism extracts of:						# lines observed in gut of VI		
A)		I	II	III	IV	V	VI	B)		Rank
Antisera to:								# lines in self-reaction		
I	8			1	3	2		I	6/8 = 75.0%	1
II	1	5			1	2	2	II	0/5 = 0	5
III	2	3	7				1	III	3/7 = 42.9	3
IV	2		1	9	6	1		IV	5/9 = 55.6	2
V		2		5	<i>10</i>	3		V	2/10 = 20.0	4
VI	1	1	1		4	<i>13</i>		VI ^a	13/13 = 100.0	—
								# lines due to cross-reactions with extracts of:		
C) Antiserum to:		# lines observed in B above		VI		I		IV		
(by rank)										
I ^d		6		—		0		0 ^b		3 = 3
IV ^d		5		—		1		2		0 = 2
III ^c		3		—		1		2		0 = 0
V ^c		2		—		3		0		5 = neg
II ^c		0		—		2		1		1 = neg

^a Not ranked since VI is the predator.^b By definition no antiserum cross-reacts with itself.^c Eliminated by algorithm due to excessive cross-reaction.^d Confirmed as prey in gut of organism VI.

(A) Cross-reaction matrix showing maximum number of precipitin lines observed in double-immunodiffusion reactions between antisera to and whole-organism extracts of organisms I–VI; italicized numbers on diagonal refer to self-reactions, and blanks denote that no precipitin line was observed; (B) Results of immunodiffusion tests on the solubilized gut contents of organism VI (the predator); the number of lines observed with each antiserum is ranked by its proportion of the appropriate self-reaction, with the highest proportion ranked first; (C) Mathematical subtraction of lines which could have been due simply to cross-reactions with other prey organisms in the gut; if the number of lines observed with an antiserum minus the numbers due to cross-reactions with other potential prey is zero or negative, then that organism is eliminated from consideration as a prey.

RESULTS

Microscopic visual examination of the mud snail gut smears revealed only the following: amorphous brown mush, amorphous green mush, golden-brown mush, pieces of pennate diatom frustules, colorless fluids, sediment, sand grains, and “detritus.” At no time were animal remains evident. All guts contained at least a little of each of the food items listed above, *i.e.*, no guts were empty. Sediments of silt and clay sizes comprised most of the volume of any individual gut, usually between 50 and 75%.

Immunoassays revealed several notable results. First, most of the precipitin line formations between gut contents and the various antisera (Table III) either were or could have been due to simple cross-reactions rather than actual presence of specific prey proteins. Second, there were no differences in gut contents between daytime low tide and nighttime low tide collections in August, 1980. Third, the absence of crustacean and living *Spartina* proteins in the guts indicates that these potential foods are not common in the mud snail diet. Fourth, meiofauna don't appear to be a major prey item, although harpacticoid copepod proteins were present in at least one snail's gut on 20 November 1981. Fifth, it is unknown whether the *Crassostrea virginica* proteins

TABLE III

Maximum number of precipitin lines observed in either of two replicate immunoassays of *Ilyanassa obsoleta* guts

Year	1980						1981			
	2 Apr	25 Aug		26 Aug		20 Nov	8 Dec	6 Jan	6 May	2 June
Time	1300	0130	1300	0200	1600	1230	1500	1430	1600	1400
<i>Antisera to:</i>										
A	1	1				1	1	1	1	
B	1					2*	1			
C										
D										
E										
F										
G										
H										1
I		1					1	6*	6*	4*
J	1	3	2	2	1	3			2	2
K	1	3	2	2	2	2	3		2	2
L	3	3	2	3	3	4	3	4	1	1
M	2	3	6	2	2	2	2	2	3	2
N	2			3			1	1		
O	14	14	14	14	14	14	14	14	14	14
P	2	2	2	2	1	2		1	2	3
Q										
R										
S							1	1		
T										

Two groups of five combined snail guts were tested each time. Blanks indicate that no lines were observed. Asterisk denotes presence of prey proteins as confirmed by algorithm of Table II. All other lines were probably due to cross-reactions as seen in Table I matrix. This data set is thus equivalent to that of Table II, part B. See Table I for antiserum identification.

present in 1981 were due to ingestion of living or dead oyster meat. Lastly, carnivory and/or carrion-feeding was not obligatory for mud snails in this size range.

DISCUSSION

These data are consistent with the hypothesis that *Ilyanassa obsoleta* is indeed a facultative carrion feeder and that herbivory and/or detritivory are the dominant feeding modes for this abundant, ecologically successful, marine deposit feeder. The visual analyses performed did not detect any semblance of carnivory, whereas the immunoassay did. Beyond this finding, however, many potential prey items that one might reasonably expect to have been present were not found in the diet of the mud snail. Chief among these were meiofaunal taxa, particularly nematodes, oligochaetes, and turbellarians. All of these taxa are abundant in the surface sediments of the high marsh where the mud snails were collected (Bell, 1979). Antisera to these meiofaunal groups can detect microgram quantities of protein, so it is unlikely that such prey would resist immunodetection if they had been present in the guts within 24 hours prior to when snail samples were collected and frozen. I take this as strong evidence that meiofauna are not a prominent dietary component of the mud snail, although Alexander (1979) found some meiofauna in the guts of periwinkle snails in a Louisiana salt marsh. Curtis and Hurd (1981) found nematodes in only a few mud snails.

Since the only *Spartina* antiserum available at the time of this study was prepared by injecting rabbits with a soluble protein extract (antigen) made from standing live green plants, the absence of any precipitin reaction with the gut contents using this antiserum was not surprising. Ingestion of living plant tissue by the snails would probably have been detected had it occurred. The presence of amorphous colored mush and detritus was likely due in part to ingestion of aged, dead *Spartina* fragments. Antisera to extracts of aged *Spartina* detritus are presently in preparation. Hopefully these will successfully distinguish among true *Spartina* detrital proteins and proteins from bacteria and fungi attached to the detritus. It will then be possible to confirm actual ingestion of detrital proteins by the snails or any other detritivore and substantiate or refute the role of attached microflora in detritus-based food webs (e.g., Newell, 1965; Adams and Angelovic, 1970).

The algorithm of Table II for confirming the presence of specific proteins is an indirect attempt to ensure that the precipitin lines observed are correctly ascribed to gut contents rather than cross-reactions. Direct evidence to confirm the presence of specific prey should be obtained by generating precipitin lines of identity between gut contents and a preparation of the actual prey protein using the antiserum specific to that particular prey. Although not done in this mud snail study, such tests in other trophic studies have established the reliability of the algorithm (pers. obs.). The algorithm does not, however, enable one to distinguish whether predators ate living or freshly dead tissue. The presence of *Crassostrea virginica* protein in snail guts in 1981 is more likely to have come from ingestion of dead oysters, as racoons frequently feed on oysters and leave debris in the high marsh. Young oysters are probably too large and thick-shelled for mud snails to penetrate. The harpacticoid copepods eaten on 20 November 1980 were also probably ingested dead, as most species present in the high marsh are mobile enough to escape the slower mud snail even though the copepods are several orders of magnitude smaller than the snails.

Robertson (1979) states that mud snails have crystalline styles and feed primarily when covered by the tide. Presence of a crystalline style, however, is not necessarily indicative of prior feeding (Curtis and Hurd, 1981). All snails had crystalline styles and were sampled within 30 min of their exposure to air at low tide. Each snail collected was also active on the sediment surface. Thus there was probably no bias introduced by inclusion in any of the samples of snails which had not recently fed.

The immunoassay as used in this study is a powerful qualitative tool for defining trophic pathways. It is limited only by the availability and sensitivity of antibodies specific to target organisms of interest. The method is not yet quantitative, however, so caution must be taken in the interpretation of its results. Only the presence, absence, or concentration of specific proteins in the predator's gut can be measured. Extrapolation of, for instance, a harpacticoid copepod protein concentration in a predator's gut to the number of copepods ingested by that predator is at present unwarranted. Rapid analysis of samples soon after collection is recommended, since proteolytic activity in the predatory snails' gut contents continues even during the 48 h incubation period of the immunodiffusion tests. Some loss of immunologically identifiable gut material may result from this proteolysis.

The mud snail's role in marine ecosystems may center on its mechanical processing of surface sediments and its associated detritus (see Conner *et al.*, 1982, and Edwards and Welsh, 1982) for it is not preyed upon by very many other organisms (Brenchley, 1982). Since Levinton and Stewart (1982) did not determine whether their mud snails actually ingested oligochaetes, the bioturbation effects of the mud snails may have been more important in the successional pattern observed than their direct biological effects. If the results of this study are indicative of its general dietary behavior in the

intertidal ecosystem, then *I. obsoleta* is probably relatively unimportant as a predator on living marine invertebrates. However, as Curits and Hurd (1981) point out, by virtue of its high numerical abundance, *I. obsoleta* could still exert considerable influence on other members of the benthic community even if it consumes small numbers of prey. As suggested by Nichols and Robertson (1979) its greatest biological effect may be one of competition with other herbivores.

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